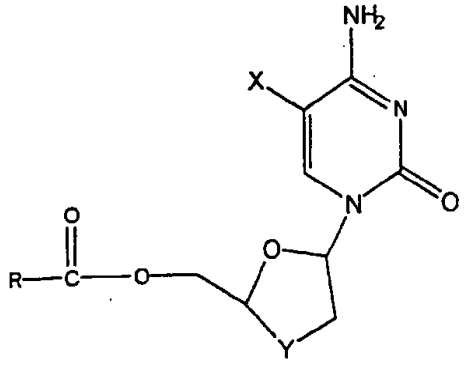
CLAIMS

We Claim:

1. A process for producing a chiral, non-racemic ester of Formula I using a hydrolase enzyme:



Formula I

wherein:

R is C_1-C_8 alkyl, alkenyl, or alkynyl;

X = H, or F;

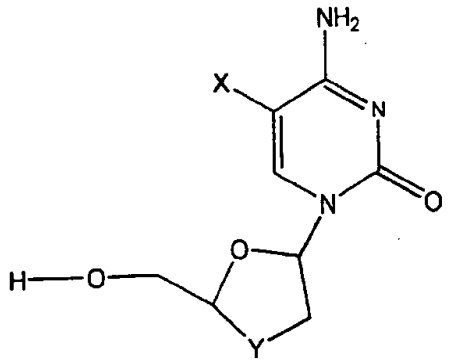
Y = CH_2, O, S, Se, or NH;

said process comprising the steps of:

(a) dispersing an enantiomeric mixture of an ester of Formula I at a concentration of between about 1 and about 25% (weight/volume of the non-homogeneous system), in an organic solvent system to produce an organic component;

(b) providing an aqueous solvent system to produce an aqueous component; and

(c) contacting said organic component and said aqueous component to form a non-homogeneous system, under conditions which permit the resolution of the mixture to produce a chiral non-racemic ester of Formula I and a non-racemic alcohol of Formula II;



Formula II

wherein:

X = H, or F;

Y = CH_2, O, S, Se, or NH, and

wherein said hydrolase enzyme is dispersed in either said organic component, said aqueous component or said non-homogeneous system.

2. A process for producing a chiral, non-racemic hydrophobic ester using a hydrolase enzyme, said process comprising the steps of:

(a) dispersing an enantiomeric mixture of said hydrophobic ester at a concentration of between about 1 and about 25% (weight/volume of the non-homogeneous system), in an organic solvent system to produce an organic component;

(b) providing an aqueous solvent system to produce an aqueous component; and

(c) contacting said organic component and said aqueous component to form a non-homogeneous system, under conditions which permit the enantioselective conversion of one enantiomeric form of said enantiomeric mixture to the corresponding alcohol; and

wherein said hydrolase enzyme is dispersed in either said organic component, said aqueous component or said non-homogeneous system.

3. A process for producing a chiral, non-racemic ester of 2-butyryloxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane using a hydrolase enzyme, said process comprising the steps of:

(a) dispersing an enantiomeric mixture of said 2-butyryloxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane at a concentration of between about 1 and about 25% (weight/volume of the non-homogeneous system), in an organic solvent system to produce an organic component;

(b) providing an aqueous solvent system to produce an aqueous component; and

(c) contacting said organic component and said aqueous component to form a non-homogeneous system, under conditions which permit the enantioselective conversion of one enantiomeric form of said enantiomeric mixture to the corresponding alcohol;

wherein said hydrolase enzyme is dispersed in either said organic component, said aqueous component or said non-homogeneous system; and

wherein the concentration of said enantiomeric mixture is calculated based on the volume of said non-homogeneous system.

4. A process for producing a chiral, non-racemic ester of 2-butyryloxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane using a hydrolase enzyme, said process comprising the steps of:

(a) dispersing an enantiomeric mixture of said 2-butyryloxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane at a concentration of between about 1 and about 25% (weight/volume of the non-homogeneous system), in an organic solvent system to produce an organic component;

(b) providing an aqueous solvent system to produce an aqueous component; and

(c) contacting said organic component and said aqueous component to form a non-homogeneous system, under conditions which permit the enantioselective conversion of one enantiomeric form of said enantiomeric mixture to the corresponding alcohol;

wherein said hydrolase enzyme is dispersed in either said organic component, said aqueous component or said non-homogeneous system;

wherein said organic component comprises between about 5 and about 90% of said non-homogeneous system;

wherein said non-homogeneous system also comprises between about 1 and about 20% of surfactant; and

wherein said surfactant concentration is calculated based on the volume of said non-homogeneous system.

5. The process according to any one of claims 1, 2, 3 or 4, wherein said hydrolase enzyme is selected from the group consisting of porcine liver esterase, porcine pancreatic lipase, *Pseudomonas species* lipase, *Aspergillus niger* lipase and subtilisin.

6. The process according to claim 5, wherein said hydrolase enzyme is a crosslinked enzyme crystal.

7. The process according to claim 6, wherein said crosslinked enzyme crystal is crosslinked with glutaraldehyde.

8. The process according to claim 5, wherein said hydrolase enzyme is an immobilized enzyme.

9. The process according to claim 5, wherein said hydrolase enzyme is a soluble enzyme.

10. The process according to claim 5, wherein said hydrolase enzyme is porcine liver esterase.

11. The process acccrding to any one of claims 1, 2, 3 or 4, wherein said chiral non-racemic ester is isolated from said organic component.

12. The process acccrding to any one of claims 1, 2, 3 or 4, wherein said chiral non-racemic alcohol is isolated frcm said aqueous component.

13. The process according to any one of claims 1 or 2, wherein said enantiomeric mixture is FTC butyrate.

14. The process according to claim 2, wherein said enantiomeric mixture comprises 2-butyryloxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane.

15. The process according to any one of claims 1, 2, 3 or 4, wherein said enantiomeric mixture is dispersed in said organic component to a concentration of between about 5% to about 15%.

16. The process according to any one of claims 1, 2, 3 or 4, wherein said enantiomeric mixture is dispersed in said organic component to a concentration of between about 1% to about 5%.

17. The process according to any one of claims 1 or 2, wherein said enantiomeric mixture is dispersed in said organic component to a concentration of between about 10% to about 20%.

18. The process according to any one of claims 1, 2, 3 or 4, wherein said organic component comprises a not more than about 50% water miscible organic solvent.

19. The process according to claim 18, wherein said organic component comprises one or more solvents selected from the group consisting of C_4-C_8 alcohols, nitromethane, dichloromethane, toluene, methyl isobutyl ketone, tert-butyl acetate and alkanes.

20. The process according to claim 19, wherein said organic component comprises one or both of n-amyl alcohol and 3-methyl-3-pentanol.

21. The process according to claim 4, wherein said surfactant is selected from the group consisting of cationic surfactants, anionic surfactants and non-ionic surfactants.

22. The process according to claim 21, wherein said surfactant is selected from the group consisting of Tween 20™, Tween 80™, Prionex™, Teepol HB7™, Tergitol TMN-6™, Tergitol TMN-10™, Tergitol NP-4™, Tergitol 15-S-3™, Igepal CA-630™, Tyloxapol™, Glucode-oxycholic acid, octyl β-gluco-pyranoside, dioctyl sulfosuccinate, and deoxycholic acid.

23. The process according to claim 22, wherein said surfactant is Tween-80™.

24. The process according to claim 22, wherein said surfactant is dioctyl sulfosuccinate.

25. The process according to claim 4, wherein said surfactant is added to said organic component.

26. The process according to claim 4, wherein said surfactant is added to said aqueous component.

27. The process according to claim 4, wherein said surfactant is added to said non-homogeneous system.

28. The process according to claim 4, wherein said surfactant is formulated with said hydrolase enzyme.

29. The process according to any one of claims 1, 2, 3 or 4, wherein said aqueous solvent system comprises water and excipients selected from the group consisting of buffering salts, alkalizing agents, anti-microbial preservatives, stabilizers, filtering aids, co-enzymes, excipients that facilitate dispersion and excipients that facilitate function of the enzyme.

30. The process according to claim 29, wherein said aqueous solvent system comprises water buffered with phosphate buffer at a pH of greater than about 7.

31. The process according to claim 29, wherein said aqueous solvent system comprises water buffered with 2-amino-2-(hydroxymethyl)-1,3-propanediol or TRIS™.

32. The process according to any one of claims 1, 2, 3 or 4, wherein said conditions which permit the enantioselective conversion of one enantiomeric form of said enantiomeric mixture to the corresponding alcohol comprise a temperature of between about 5°C and about 45°C.

33. A non-homogeneous system for producing a chiral, non-racemic hydrophobic ester using a hydrolase enzyme, comprising:

(a) a hydrolase enzyme;

(b) a hydrophobic ester substrate;

(c) an organic component; and

(d) an aqueous component.

34. The non-homogeneous system according to claim 33, wherein said hydrolase enzyme is selected from the group consisting of porcine liver esterase, porcine pancreatic lipase, *Pseudomonas species* lipase, *Aspergillus niger* lipase and subtilisin.

35. The non-homogeneous system according to claim 33, wherein said hydrolase enzyme is a crosslinked enzyme crystal.

36. The non-homogeneous system according to claim 35, wherein said crosslinked enzyme crystal is crosslinked with glutaraldehyde.

37. The non-homogeneous system according to claim 33, wherein said hydrolase enzyme is an immobilized enzyme.

38. The non-homogeneous system according to claim 33, wherein said hydrolase enzyme is a soluble enzyme.

39. The non-homogeneous system according to claim 34, wherein said hydrolase enzyme is porcine liver esterase.

40. The non-homogeneous system according to claim 33, wherein said hydrophobic ester substrate is an enantiomeric mixture.

41. The non-homogeneous system according to claim 40, wherein said enantiomeric mixture comprises 2-butyryloxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane.

42. The non-homogeneous system according to claim 40, wherein said enantiomeric mixture is dispersed in said organic component to a concentration of between about 5% to about 15%.

43. The non-homogeneous system according to claim 40, wherein said enantiomeric mixture is dispersed in said organic component to a concentration of between about 10% to about 20%.

44. The non-homogeneous system according to claim 40, wherein said enantiomeric mixture is dispersed in said organic component to a concentration of between about 1% to about 5%.

45. The non-homogeneous system according to claim 33, wherein said organic component comprises a not more than about 50% water miscible organic solvent.

46. The non-homogeneous system according to claim 45, wherein said not more than about 50% water miscible organic solvent comprises one or more solvents selected from the group consisting of C_4-C_8 alcohols,

nitromethane, dichloromethane, toluene, methyl isobutyl ketone, tert-butyl acetate and alkanes.

47. The non-homogeneous system according to claim 46, wherein said organic component comprises one or both of n-amyl alcohol and 3-methyl-3-pentanol.

48. The non-homogeneous system according to claim 33, further comprising a surfactant.

49. The non-homogeneous system according to claim 48, wherein said surfactant is selected from the group consisting of cationic surfactants, anionic surfactants and non-ionic surfactants.

50. The non-homogeneous system according to claim 49, wherein said surfactant is selected from the group consisting of Tween 20™, Tween 80™, Prionex™, Teepol HB7™, Tergitol TMN-6™, Tergitol TMN-10™, Tergitol NP-4™, Tergitol 15-S-3™, Igepal CA-630™, Tyloxapol™, Glucode-oxycholic acid, octyl β-glucopyranoside, dioctyl sulfosuccinate, or deoxycholic acid.

51. The non-homogeneous system according to claim 50, wherein said surfactant is Tween-80™.

52. The non-homogeneous system according to claim 50, wherein said surfactant is dioctyl sulfosuccinate.

53. The non-homogeneous system according to claim 48, wherein said organic component comprises said surfactant.

54. The non-homogeneous system according to claim 48, wherein said aqueous component comprises said surfactant.

55. The non-homogeneous system according to claim 48, wherein said surfactant is formulated with said hydrolase enzyme.

56. The non-homogeneous system according to claim 33, wherein said aqueous solvent system comprises water and excipients selected from the group consisting of buffering salts, alkalizing agents, anti-microbial preservatives, stabilizers, filtering aids, co-enzymes, excipients that facilitate dispersion and excipients that facilitate function of the enzyme.

57. The non-homogeneous system according to claim 33, wherein said aqueous solvent system comprises water buffered with phosphate buffer at a pH of greater than about 7.

58. The non-homogeneous system according to claim 33, wherein said aqueous component comprises water buffered with 2-amino-2-(hydroxymethyl)-1,3-propanediol (TRIS™) at a pH of greater than about 7.

59. The non-homogeneous system according to claim 33, wherein said organic component and said aqueous component are contacted under conditions which permit the enantioselective conversion of one enantiomeric form of said enantiomeric mixture to the corresponding alcohol.

60. The non-homogeneous system according to claim 59, wherein said organic component and said aqueous component are contacted under conditions which permit the enantioselective conversion of one

enantiomeric form of said enantiomeric mixture to the corresponding alcohol, comprise a temperature of between about 5°C and about 45°C.